

ASSESSMENT

from Prof. Dr. Eng. Dancho Lyubenov Danalev

University of Chemical Technology and Metallurgy, Sofia, Biotechnology
department

Regarding: awarding of the Ph.D. degree to Nikola Nikolov Atanasov, in the field of higher education 5. Technical sciences, professional direction 5.11. Biotechnologies, Doctoral program: Technology of biologically active substances

Topic of the Ph.D. thesis: *"Properties and characteristics of newly isolated strains of lactic acid bacteria and application in model probiotic products for oral health"*

Headed by: Assoc. Prof. D-r Dilyana Nikolova

1. Main data for the candidate

The candidate for obtaining the Ph.D. Nikola Nikolov Atanasov obtained the first degree of higher education "Professional Bachelor" with the professional qualification dental technician at the Medical College of Medical University-Sofia. Further, he graduated his bachelor and master degrees in the Faculty of Biology of the Sofia University "St. Kliment Ohridski" in the specialties Biotechnology and Industrial biotechnology, respectively. All this logically predetermines his development as a doctoral student in the "Biotechnology" department of the Sofia University "St. Kliment Ohridski", and later his realization as an assistant in the same department. The choice of scientific direction and topic of his Ph.D. thesis work is also logical, which are undoubtedly a deepening of the knowledge, skills and competences acquired during his education.

2. Relevance and significance of the dissertation topic

Oral hygiene is a part of every person's daily life and includes keeping the oral cavity clean to prevent the occurrence of problems such as various diseases of both the teeth and mucous membranes in the mouth, bad breath, etc. The most common types of disease are dental caries and gum disease, including gingivitis and periodontitis. Dental caries is the most common oral disease worldwide, affecting millions of people, including children and adults. It is a relatively slowly progressing process, which allows to be prevented or influenced and stopped when it occurs. In this context, the use of probiotic products for the prevention of oral health is a widely distributed concept in practice, which makes the developed topic relevant and directly related to the needs of dental medicine. In the literature, there are new data on the application of different approaches, including natural products such as peptides and proteins, possessing specific self-assembly properties, which help to reverse the process of the appearance of early carious

lesions and the remineralization of tooth enamel. All this shows the exceptional relevance of the topic chosen and developed by the doctoral student.

3. Fulfillment of the minimum state requirements and the individual plan for the development of the Ph.D. work

From the submitted documents, it is clear that Ph.D. student Nikola Nikolov Atanasov is enrolled as a doctoral student in the Department of Biotechnology of Sofia University "St. Kliment Ohridski" in 2020 (Order RD-20-1009/16.07.2020) and was dismissed with the right of defense in 2023 (Order RD-20-1694/28.09.2023), as during the period while developing his Ph.D. thesis, he successfully passed all the exams planned in his individual plan. As a result of the Ph.D. thesis work, the candidate for the Ph.D. degree presents three scientific publications in journals referenced in the world databases Scopus and WoS, which bring him a total point asset of **37** points, thus fully satisfying the requirements of a minimum of **30** points for the Regulations for application of the act for development of the academic staff of the Republic of Bulgaria. A search in the Scopus database shows available 5 citations of the articles presented in the Ph.D. thesis, which form an *h-factor* of **3**, which is an extremely good certificate for the recognition of the research that Nikola Atanasov is engaged in among the scientific community. It is noteworthy that the candidate participated in **6** projects on the subject of the dissertation. The results of the Ph.D. thesis have been reported at **13** national and international scientific conferences with posters and one plenary report. All this undoubtedly gave him additional scientific and administrative experience to summarize, analyze and interpret obtained scientific data.

4. Evaluation of the structure of Ph.D. thesis

The Ph.D. thesis is developed on 149 standard pages, A4 format, containing the following parts: Introduction - 1 page, Literature review on the researched topic - 37 pages, Aim and tasks - 2 pages, Materials and methods - 12 pages, Results and discussion – 47 pages, Conclusions – 2 pages, Contributions – 1 page and References – 34 pages. The material and results in the dissertation work are systematized in **18** tables and **26** figures. Thus, the development is very well visualized, and the obtained data are summarized in a concise form and allow to follow the implementation of the main tasks set in the Ph.D. thesis. The literature review is concise, but at the same time, a large number of scientific papers covering the Human Oral Microbiome, Microbial balance and imbalance in the oral cavity and its relationship to oral and systemic health is analyzed, paying attention to the various diseases that can occur in imbalance of the normal environment in the oral cavity. In addition, the specific characteristics of probiotic microorganisms and lactic acid bacteria, as well as the mechanisms of influencing the oral microbiome, were examined and analyzed. At the end of the literature review, special attention is paid to the oral health products available in practice. The literature

review is impressively based on 467 literary sources, all of which are after the year 2000, and the main part are from recent years, which is another certificate for the relevance of the chosen topic. All this completely exhausts the necessary prerequisites for setting the Goals and tasks of the dissertation work, which are set precisely, clearly and concisely, which makes it easier to track their implementation in the development process.

The Results and Discussion section includes a large experimental work related to:

- Isolation and characterization of new strains of lactic acid bacteria from human oral microbiome samples and determination of their main morphological and physiological characteristics;
- Screening for a wide range of enzymes activities of the isolated lactic acid bacteria;
- Taxonomic characterization of the newly isolated strains;
- Evaluation of the survival and development dynamics of the investigated strains under simulated conditions of the different parts of the gastrointestinal tract;
- Assessment of adhesive potential and antibiotic resistance against a wide range of the most widely prescribed antibiotics;
- Antioxidant potential screening;
- Antagonistic activity with microbial test-pathogens, including oral;
- Survival of the studied strains of lactic acid bacteria during the lyophilization and storage process;
- Stability and survival of a selection of the investigated strains in different formulations of a model oral health product.

The experimental conditions are described in full detail to allow their subsequent use in similar studies. An excellent impression is made by the fact that all obtained results were considered in the context of results obtained by other scientific groups, and various conclusions and relationships were drawn regarding the degree of usefulness of the obtained results both from a scientific point of view and for practice. The Ph.D. thesis is presented in a high scholarly style that shows a good understanding of the subject matter. Based on the conducted experimental work, 13 conclusions were formulated, arising logically from the obtained results and providing accurate information about the value of the conducted experiments. Original scientific and applied contributions are highlighted, such as the isolation of 12 new strains from the human oral microbiome, which have been fully characterized and identified to species by applying a modern combined molecular approach, the genomes of the 12 newly isolated strains have been sequenced, and the genomic sequences have been deposited in the NCBI genetic database, a complex approach adapted according to the tasks set in the Ph.D. thesis was applied to evaluate the probiotic potential of the newly isolated strains of lactic acid bacteria by applying a newly structured point system and new model probiotic products were formulated with the successful inclusion of two selected strains of *L. fermentum* N 2 and *L. delbrueckii subsp. lactis* VG 2.

I have no critical remarks about the Ph.D. and I consider that it was developed at a high scientific level.

5. Assessment of correspondence between the abstract and the Ph.D. thesis

The presented abstract fully reflects all the important highlights of the scientific research and the obtained results. It is designed strictly according to the requirements of the Regulations for application of the act for development of the academic staff of the Republic of Bulgaria.

6. Conclusion

Based on all of the above, it can be concluded that the Ph.D. thesis presented for public defense is a precisely conducted experimental work, based on a thorough analysis of modern data in the literature, as well as an excellent and competent interpretation of the obtained results and comparison with data from other scientific developments. All this has allowed the candidate for the Ph.D. degree Nikola Nikolov Atanasov to deepen his knowledge, skills and competences in a basic area of Biotechnology, namely microbiology, as well as in related areas such as pharmaceutical biotechnology, applying a multidisciplinary approach to achieve the set goals and tasks. The topicality of the topic for science and practice is also undoubted, as the educational and scientific activity of the Ph.D. student, as well as the volume of research and the achieved scientific contributions, fully satisfy the requirements of the Act for development of the academic staff of the Republic of Bulgaria, the Regulations for its application and the Regulations of Sofia University "St. Kl. Ohridski" for the acquisition of the Ph.D. degree. Taking into account everything set forth in this opinion, I am convinced to vote **positively** for awarding the Ph.D. degree in the field of higher education 5. Technical sciences, professional direction 5.11. Biotechnologies, Doctoral program: Technology of biologically active substances to Nikola Nikolov Atanasov.

Sofia, 08.07.2024 г.

Prof. Dr. Eng. Dancho Danalev: